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Myostatin dysfunction impairs force generation in extensor digitorum longus muscle and increases exercise-induced protein efflux from extensor digitorum longus and soleus muscles

Baltusnikas, J; Kilikevicius, A; Venckunas, T; Fokin, A; Bunger, L; Lionikas, A; Ratkevicius, A

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Myostatin dysfunction impairs force generation in extensor digitorum longus muscle and increases exercise-induced protein efflux from soleus muscle

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1 **Myostatin dysfunction impairs force generation in *extensor digitorum longus* muscle and**
2 **increases exercise-induced protein efflux from *extensor digitorum longus* and *soleus***
3 **muscles**

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5 Juozas Baltusnikas¹, Audrius Kilikevicius¹, Tomas Venckunas¹, Andrej Fokin¹, Lutz
6 Bünger³, Arimantas Lionikas², Aivaras Ratkevicius^{1,2}.

7
8 ¹Institute of Sports Sciences and Innovation, Lithuanian Sports University, Kaunas, Lithuania

9 ²School of Medical Sciences, College of Life Sciences and Medicine, University of
10 Aberdeen, Aberdeen, Scotland, UK

11 ³Scotland's Rural College (SRUC), Edinburgh, UK

12
13 **Correspondence to:**

14 Juozas Baltušnikas, MSc

15 Institute of Sports Sciences and Innovation, Lithuanian Sports University,
16 Sporto 6, LT-44221, Kaunas, Lithuania

17 Phone: +370 671 00819; Fax: +370 37 204 515, E-mail: juozas.baltusnikas@lsu.lt

18
19 E-mails:

20 Kilikevicius Audrius: audrius.kilikevicius@lsu.lt

21 Venckunas Tomas: tomas.venckunas@lsu.lt

22 Fokin Andrej: fokinandrej@yahoo.com

23 Lutz.Bunger@sruc.ac.uk

24 Lionikas Arimantas: a.lionikas@abdn.ac.uk

25 Ratkevicius Aivaras: a.ratkevicius@abdn.ac.uk

26 **ABSTRACT**

27 Myostatin dysfunction promotes muscle hypertrophy which can complicate assessment of
28 muscle properties. We examined force generating capacity and creatine kinase (CK) efflux
29 from skeletal muscles of young mice before they reach adult body and muscle size. Isolated
30 *soleus* (SOL) and *extensor digitorum longus* (EDL) muscles of Berlin high (BEH) mice with
31 dysfunctional myostatin, i.e. homozygous for inactivating myostatin mutation, and with a
32 wild type myostatin (BEH+/+) were studied. The muscles of BEH mice showed faster ($P <$
33 0.01) twitch and tetanus contraction times compared to BEH+/+ mice, but only EDL
34 displayed lower ($P < 0.05$) specific force. SOL and EDL of age matched, but not younger
35 BEH mice showed greater exercise-induced CK efflux compared to BEH+/+ mice. In
36 summary, myostatin dysfunction leads to impairment in muscle force generating capacity in
37 EDL and increases susceptibility of SOL and EDL to protein loss after exercise.

38
39 **Keywords:** lengthening contractions, muscle force, muscle damage, myostatin.

40

41 INTRODUCTION

42 Skeletal muscles play an important role in health and disease (Wolfe 2006). Unaccustomed
43 exercise and some diseases can lead to injury and efflux of proteins from the affected muscles
44 (Armstrong 1984). An increase in total plasma CK activity has been used as evidence of
45 muscle damage after exercise in humans (Brancaccio et al. 2007; Skurvydas et al. 2011).
46 However, swelling and infiltration of skeletal muscles by immune cells can occur without
47 signs of structural damage after exercise (Pizza et al. 2002, Yu et al. 2013). It is believed that
48 exercise increases permeability of sarcolemma and can trigger the secondary events
49 associated with actions of immune system (Tidball 1995; McHugh 2003, Yu et al. 2013).
50 Isolated skeletal muscle model permits studying the primary effects of exercise by limiting
51 the complex influence of the immune and hormonal systems (Jackson et al. 1987; Suzuki et
52 al. 1999).

53
54 Various hormones and growth factors can affect functional properties and susceptibility to
55 damage of the skeletal muscles (Amelink et al. 1990). There has been a considerable interest
56 in effects of myostatin (Smith and Lin 2013). Myostatin knockout is associated with a
57 significant increase in muscle mass due to muscle fiber hypertrophy and hyperplasia
58 (McPherron et al. 1997). Myostatin blockade can improve muscle function in Duchenne
59 muscular dystrophy (Bogdanovich et al. 2002), and has been proposed as a promising
60 treatment strategy against muscle wasting in chronic diseases (Grossmann et al. 2014).
61 However, myostatin dysfunction has also been associated with low specific force of skeletal
62 muscles (Amthor et al. 2007; Matsakas et al. 2010). Interestingly, endurance training can lead
63 to normalization of specific muscle force in myostatin null mice (Matsakas et al. 2012). Food
64 restriction was also associated with an increase in specific muscle force of these mice
65 (Matsakas et al. 2013). Both endurance training and food restriction caused a reduction in

muscle mass, which might improve intramuscular force transmission. Furthermore, myostatin dysfunction is also associated with a shift in muscle fiber composition towards faster contracting fiber types (Amthor et al. 2007). Type 2 muscle fibers characterized by a faster contraction time and are more sensitive to exercise-induced muscle damage than slow contracting type 1 fibers (Macaluso et al. 2012; Chapman et al. 2013). Thus myostatin inhibition may increase susceptibility of skeletal muscles to damage (Mendias et al. 2006).

It appears that myostatin effects are complex, vary between the skeletal muscles and can be further complicated by excessive muscle hypertrophy. The aim of our study was to examine effects of myostatin dysfunction on contractile properties and CK efflux in skeletal muscles of young mice before they reached adult body and muscle size. We have studied *extensor digitorum longus* (EDL) and *soleus* (SOL) muscles from Berlin high (BEH) mice with mutant myostatin, known as *compact* allele, and the wild type myostatin allele (BEH^{+/+}) (Amthor et al. 2007; Lionikas et al. 2013). The BEH and BEH^{+/+} mice were matched by muscle mass to minimize the influence of excessive muscle hypertrophy as a possible confounding factor.

MATERIALS AND METHODS

Animals and experiments

All procedures of this experiment were approved by the Lithuanian State Food and Veterinary Service (Nr. 0223). BEH^{+/+} females were generated by crossing animals from BEH and Berlin Low (BEL) strains and then repeatedly backcrossing the offspring to BEH using marker assisted selection for the wild type myostatin (Amthor et al. 2007; Lionikas et al. 2013). The data on age, body mass and muscle mass of these animals are presented in Table 1. BEH mice were younger than BEH^{+/+} mice when matched by the muscle mass of SOL or EDL. The age difference between the strains was particularly significant in case of

91 EDL. Thus additional measurements were carried out on EDL of BEH mice of a similar age
92 as BEH+/+ mice. Prior to the *in vitro* experiments, animals were kept in standard cages (cage
93 dimensions: 267 x 207 x 140 mm) at 20-22° C temperature and 55±10% humidity with 12/12-
94 h light/dark cycle. As in our previous studies (Kilikevicius et al. 2013, Lionikas et al. 2013),
95 mice were housed one to three mice per cage, fed standard rodent diet (58.0 % kcal from
96 carbohydrates, 28.5 % kcal from protein, 13.5 % kcal from fat; LabDiet 5001, LabDiet, St.
97 Louis, USA) and received tap water *ad libitum*.

98 **Muscle properties and CK efflux**

99 All experiments were performed at room temperature (~25 °C). Mice were euthanized by the
100 cervical dislocation. Afterwards, SOL or EDL muscle of the right leg was dissected, freed
101 from tendons, blotted and weighed (Kern, ABS 80-4, Germany). Muscles of the left leg were
102 used for assessment of contractile properties and total muscle CK efflux as described
103 previously (Baltusnikas et al. 2014). The muscles were dissected and placed immediately in
104 the organ bath containing Tyrode solution (121 mM NaCl, 5 mM KCl, 0.5 mM MgCl₂, 1.8
105 mM CaCl₂, 0.4 mM NaH₂PO₄, 0.1 mM NaEDTA, 24 mM NaHCO₃, 5.5 mM glucose) which
106 was bubbled with 95 % O₂ and 5 % CO₂ to attain a pH of ~7.4. Muscles were fixed between
107 two platinum plate electrodes of the muscle test system (1200A-LR Muscle Test System,
108 Aurora Scientific Inc., Aurora, Canada). Then the muscle length was increased progressively
109 in steps until peak force was reached in 150-Hz tetani of 0.5-s or 2-s duration which were
110 induced every 2 min in EDL or SOL, respectively. Single stimulus was then delivered to
111 assess twitch contraction time and 90% twitch relaxation time, and this was followed by a
112 measurement of peak tetanic force as well as 90% contraction and relaxation times. Then
113 muscles were subjected to 100 eccentric contractions at a frequency of 0.1 Hz. During the
114 exercise, muscles were stimulated at 150 Hz stimulation for 700 ms. During the last 200 ms
115 of this stimulation a ramp stretch was performed followed by 200 ms gradual return of the

muscle to the initial length without any stimulation. The amplitude of the stretch was equivalent to 2.5 fiber lengths per second in case of both SOL and EDL muscles (Brooks and Faulkner 1988). After the eccentric exercise muscles were photographed with the length scale in the background for assessment of optimal muscle length (L_0). Then these muscle as well as muscles from the control experiment without any exercise, were incubated in 2 ml of Tyrode solution for 2 h at room temperature. 250 μ l of Tyrode solution was sampled for assessment of CK activity using biochemical analyser (SpotchemTM EZ SP-4430, Menarini Diagnostics, UK) with the reagent strips (Arkray Factory, Inc., Shiga, Japan).

The physiological cross-sectional areas (pCSA) of SOL and EDL were estimated by dividing muscle weight by the product of fiber length and 1.06 kg l^{-1} , the density of mammalian skeletal muscle (Brooks and Faulkner 1988). Muscle fiber length was assumed to be equal to 45% and 70% of muscle length for EDL and SOL, respectively. Muscles tended to show a slight increase in weight after the experimental protocol involving repetitive exercise. Thus weights of the contralateral muscles were used in these assessments. In a large set of samples ($n=101$) we dissected both left and right solei of adult mice to immediately measure wet muscle mass; there was no difference (paired t-test $p=0.953$) found in weights of the contralateral muscles.

Statistical analysis

All data analysis was performed using Prism 5.0 software. Data for SOL and EDL were analyzed separately. The two factor analysis of variance (ANOVA) was used to assess effects of experimental intervention (exercise or rest) and mouse strain (BEH $^{+/+}$ or BEH) on muscle CK efflux. Repeated measures ANOVA was used for the analysis of peak isometric force during eccentric exercise. The post hoc testing was carried out using t-tests with a Bonferroni correction for multiple comparisons. Non parametric Mann–Whitney U test was used in all other cases. All the tests were two-tailed with significance level was set at $P < 0.05$.

RESULTS

Data on muscle properties of BEH^{+/+} and BEH mice are presented in Table 2. There were no strain differences for SOL muscle. However, strain effects were found in EDL. BEH^{+/+} mice had a longer L_0 ($P < 0.01$) and a smaller ($P < 0.01$) pCSA of EDL compared to BEH mice. The older BEH mice showed the greatest pCSA ($P < 0.01$) of this muscle. In spite of greater pCSA, EDL of young BEH generated less force ($P < 0.05$) and showed a lower ($P < 0.01$) specific force compared to BEH^{+/+}. The older BEH mice had the highest ($P < 0.01$) peak force for EDL, but their specific force was similar as in young BEH mice and lower ($P < 0.01$) compared to BEH^{+/+} mice.

The contraction speed of a single twitch and tetanus of the muscles from BEH^{+/+} and BEH mice are presented in Table 2. For SOL, BEH mice had shorter contraction times in single twitch ($P < 0.01$) and 150-Hz tetani ($P < 0.01$) than BEH^{+/+} mice. Data for the EDL were less consistent than for SOL. BEH mice had longer contraction times ($P < 0.01$), but shorter ($p < 0.05$) relaxation times in single twitches compared to BEH^{+/+} mice. The opposite was true for tetani. Relaxation times were longer ($P < 0.01$) for BEH mice compared to BEH^{+/+}. Only older, but not younger BEH mice showed shorter ($p < 0.05$) contraction times of tetanus than BEH^{+/+} mice.

Data on peak isometric force for SOL and EDL during repeated isometric-eccentric exercise are shown in Fig. 1. BEH^{+/+} and young BEH mice showed similar loss ($P < 0.001$) of peak isometric force for both muscles during the exercise. For EDL, older BEH mice showed a greater ($P < 0.05$ - 0.01) decline in isometric force compared to both young BEH and BEH^{+/+}

mice after initial ten and twenty contractions, respectively. Afterwards, however, the relative decline of peak isometric force was similar in all mice.

Data on the total CK efflux from the muscles of mice are presented in Fig. 2. There were no differences between the strains in muscle CK efflux when measurements were performed at rest, i.e. without prior exercise. After the exercise muscle CK efflux increased ($P < 0.05$ - 0.01) and younger BEH mice showed a greater ($P < 0.05$) CK efflux from SOL compared to BEH $^{+/+}$ mice. There were no differences between these mice for the EDL. However, older BEH mice showed a greater ($P < 0.05$) CK efflux from EDL compared to the age-matched BEH $^{+/+}$ and younger BEH.

DISCUSSION

The aim of the study was to examine the effects of myostatin dysfunction on the contractile properties and total CK efflux from SOL and EDL muscles at rest and after exercise. The results of the study show that BEH mice with myostatin dysfunction had lower specific force than BEH $^{+/+}$ mice with the wild type myostatin in the faster contracting EDL, but not in the slower contracting SOL. Furthermore, BEH mice demonstrated greater exercise-induced muscle CK efflux compared to BEH $^{+/+}$ when mice of similar age were compared, but not at young age. These results show that effects of myostatin dysfunction vary between skeletal muscles and depend on the age of mice.

Myostatin dysfunction is associated with excessive muscle hypertrophy (McPherron et al. 1997) and reduction in specific muscle force of the fast contracting EDL (Amthor et al. 2007; Mendias et al. 2006). It has been hypothesized that enlargement of muscle fibers might impair force transmission within the skeletal muscles due to an increase in muscle fiber

190 pennation angles (Amthor et al. 2007). However, muscle fibers of myostatin null mice might
191 also show **an** intrinsic reduction in force output due to **a** low content of contractile proteins
192 (Qaisar et al. 2012). **We studied skeletal muscles of young mice before they developed**
193 **excessive muscularity.** This approach **minimized** confounding effects of muscle hypertrophy.
194 Nevertheless, **EDL of BEH mice showed lower specific force compared to BEH+/+ mice in**
195 **both cases, i.e. when muscles were matched by weight or age.** Thus impairment in force
196 generating capacity of EDL muscle in myostatin deficient mice was independent of muscle
197 size, and appears to be due to reduced force output at the level of single muscle fibers (Qaisar
198 et al. 2012). Interestingly, BEH+/+ and BEH mice did not differ in the specific force of SOL
199 muscle. Similar findings on the differences between EDL and SOL muscles have been
200 reported for adult mice (Mendias et al. 2006). Endurance training can improve specific force
201 of skeletal muscles in myostatin null mice (Matsakas et al. 2012). It might be that motor
202 activity helps to maintain specific force of SOL in BEH mice in spite of myostatin deficiency.
203 **SOL muscle shows markedly greater involvement in locomotion than other leg muscles**
204 **which prevail in daily activity of mice including EDL (Roy et al. 1991).**
205
206 BEH mice showed shorter contraction times in both single twitches and tetani of SOL
207 compared to BEH+/+ mice. This might be associated with a shift in muscle fiber composition
208 towards faster contracting fiber types **in SOL muscle of mice with myostatin deficiency**
209 **compared to the wild type mice** (Girgenrath et al. 2005; Amthor et al. 2007; Matsakas et al.
210 2010). **Fast twitch muscle fibers of mice and humans are more susceptible to damage after**
211 **eccentric exercise than slow twitch muscle fibers (Mendias et al. 2006; Chapman et al. 2013).**
212 Indeed, SOL muscle of BEH mice showed greater CK efflux after exercise compared to
213 BEH+/+ mice.
214

Effects of myostatin dysfunction were less consistent for EDL than SOL. This might be associated with differences in myostatin effects on fiber type composition of EDL and SOL. Myostatin dysfunction causes a marked increase in content of 2X and 2B fibers at the expense of type 1 fibers in SOL, but induces only a small increase in content of type 2B fibers at the expense of type 2X fibers in EDL (Girgenrath et al. 2005). Age of the studied mice might also be of importance here. BEH mice of similar age as BEH+/+ mice, but not young BEH mice showed elevated CK efflux from EDL after eccentric exercise compared to BEH+/+ mice. A study by Gokhin et al. 2008 demonstrated that contractile force, fiber cross-sectional area, area of the fibers occupied by the contractile proteins, and percentage of type 2B fibers increase rather drastically in mouse tibialis anterior muscle between day 1 and day 21 after birth. Then the changes between day 21 and day 28 are much more subtle. For instance, area of the fibers occupied by the contractile proteins – the most relevant index in relation to the specific force, does not change between these time points; and proportion of type 2B fibres is comparable to that of the adult animals (Bloemberg & Quadriatello 2012) already at the age of 21 days. Because young BEH mice were at 26 days of age and BEH+/+ at 37 days, both have already passed the phase where developmental differences might had played a sizable role. However, muscle resistance to exercise-induced protein efflux is dependent on other factors than specific force. Collagen content of extracellular matrix might be of particular importance here. Procollagen processing increases after eccentric exercise in both rats and humans (Han et al. 1999; Crameri et al. 2004). Myostatin belongs to transforming growth factor (TGF- β) family of cytokines that signal through Smad2/3, TAK1-p38 MAPK pathways (Lee 2004; Tsukada et al. 2005). Inhibition of TGF- β signaling suppresses collagen expression in EDL of mice after injury (Gumucio et al. 2013). It could be that concentration and/or properties of structural proteins become insufficient to sustain high mechanical loads during the phase of rapid muscle growth between 26 and 40 days and

susceptibility to exercise-induced muscle damage increases in mice with myostatin dysfunction.

We did not observe any significant difference in loss of peak isometric force between BEH and BEH^{+/+} muscles during eccentric exercise. Muscle contractions were separated by 10 s periods of rest that should minimize metabolic inhibition during the exercise (Allen et al. 1995). Our exercise protocol included stretches of similar amplitude, but at half of the velocity compared to the previous study of myostatin effects on muscles of adult (10-12 month old) mice (Mendias et al. 2006). In general, exercise-induced CK efflux from skeletal muscles is not always associated with changes in muscle force. Eccentric contractions often induce an impairment in excitation-contraction coupling of muscle fibers without any clear sign of muscle damage (Warren et al., 1993, Allen, 2001). Such impairment will lead to inactivation of muscles fibers and will protect them from damaging effects of exercise. It appears that changes in force generating capacity can be dissociated from alterations in permeability of sarcolemma and muscle CK efflux during and after exercise. Indeed, the regenerated SOL muscle showed a particularly low exercise-induced CK efflux in spite of relatively modest improvement in fatigue resistance compared to the control muscles (Baltusnikas et al. 2014).

In summary, myostatin dysfunction leads to impairment in muscle force generating capacity of faster contracting EDL and increased susceptibility of both SOL and EDL to protein efflux after eccentric exercise.

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CONFLICTS OF INTEREST

We declare that we have no conflict of interests.

REFERENCES

- Allen, D.G., Lännergren, J., and Westerblad, H. 1995. Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Exp. Physiol.* **80**(4): 497-527.
- Amelink, G.J., Koot, R.W., Erich, W.B., Van Gijn, J., and Bär, P.R. 1990. Sex-linked variation in creatine kinase release, and its dependence on oestradiol, can be demonstrated in an *in vitro* rat skeletal muscle preparation. *Acta Physiol. Scand.* **138**(2): 115-24.
- Amthor, H., Macharia, R., Navarrete, R., Schuelke, M., Brown, S.C., Otto, A., et al. 2007. Lack of myostatin results in excessive muscle growth but impaired force generation. *Proc. Natl. Acad. Sci. U.S.A.* **104**(6): 1835-40.
- Armstrong, R.B. 1984. Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Med. Sci. Sports Exerc.* **16**(6): 529-38.
- Baltusnikas, J., Kilikevicius, A., Venckunas, T., Fokin, A., Lionikas, A., and Ratkevicius, A. 2014. Regenerated *soleus* muscle shows reduced creatine kinase efflux after contractile activity *in vitro*. *Appl. Physiol. Nutr. Metab.* **10**: doi:10.1139/apnm-2014-0274.
- Bogdanovich, S., Krag, T.O., Barton, E.R., Morris, L.D., Whittemore, L.A., Ahima, R.S., et al. 2002. Functional improvement of dystrophic muscle by myostatin blockade. *Nature.* **420**(6914): 418-21.

- 288 Bloemberg, D., and Quadrilatero J. 2012. Rapid determination of myosin heavy chain
289 expression in rat, mouse, and human skeletal muscle using multicolor immunofluorescence
290 analysis. PLoS One. 7(4):e35273. doi: 10.1371/journal.pone.0035273.
- 291 Brancaccio, P., Maffulli, N., and Limongelli, F.M. 2007. Creatine kinase monitoring in sport
292 medicine. Br. Med. Bull. **81-82**: 209-30.
- 293 Brooks, S.V., and Faulkner, J.A. 1988. Contractile properties of skeletal muscles from young,
294 adult and aged mice. J. Physiol. **404**: 71-82.
- 295 Chapman, D.W., Simpson, J.A., Iscoe, S., Robins, T., and Nosaka, K. 2013. Changes in
296 serum fast and slow skeletal troponin I concentration following maximal eccentric
297 contractions. J. Sci. Med. Sport. **16**(1): 82-5. doi:10.1016/j.jsams.
- 298 Crameri, R.M., Langberg, H., Teisner, B., Magnusson, P., Schröder, H.D., Olesen, J.L.,
299 Jensen, C.H., Koskinen, S., Suetta, S.C., and Kjaer, M. 2004. Enhanced procollagen
300 processing in skeletal muscle after a single bout of eccentric loading in humans. Matrix Biol.
301 **23**:259–264.
- 302 Han, X.-Y., Wang, W., Komulainen, J., Koskinen, S.O.A., Kovanen, V., Vihko, V.,
303 Trackman, P.C., and Takala, T.E.S., 1999. Increased mRNAs for procollagens and key
304 regulating enzymes in rat skeletal muscle following downhill running. Pflugers Arch. 437,
305 857– 864.
- 306 Girgenrath, S., Song, K., and Whittemore, L.A. 2005. Loss of myostatin expression alters
307 fiber-type distribution and expression of myosin heavy chain isoforms in slow- and fast-type
308 skeletal muscle. Muscle Nerve. **31**(1): 34-40.
- 309 Gokhin, D.S., Ward, S.R., Bremner, S.N., Lieber, R.L. 2008. Quantitative analysis of
310 neonatal skeletal muscle functional improvement in the mouse. J. Exp. Biol. 211(Pt 6):837-
311 43. doi: 10.1242/jeb.014340.

- 312 Grossmann, M. 2014. Myostatin inhibition: a new treatment for androgen deprivation-
313 induced sarcopenia?. *J. Clin. Endocrinol. Metab.* **99**(10): 3625-8. doi:10.1210/jc.2014-3290.
- 314 Gumucio JP, Flood MD, Phan AC, Brooks SV, Mendias CL. 2013. Targeted inhibition of
315 TGF- β results in an initial improvement but long-term deficit in force production after
316 contraction-induced skeletal muscle injury. *J. Appl. Physiol.* **115**: 539–545
- 317 Jackson, M.J., Wagenmakers, A.J., and Edwards, R.H. 1987. Effect of inhibitors of
318 arachidonic acid metabolism on efflux of intracellular enzymes from skeletal muscle
319 following experimental damage. *Biochem. J.* **241**: 403-407.
- 320 Kilikevicius, A., Venckunas, T., Zelniene, R., Carroll, A.M., Lionikaite, S., Ratkevicius, A.,
321 and Lionikas, A. 2013. Divergent physiological characteristics and responses to endurance
322 training among inbred mouse strains. *Scand. J. Med. Sci. Sports*, **23**(5): 657-68.
323 doi:10.1111/j.1600-0838.2012.01451.
- 324 Lee, SJ. 2004. Regulation of muscle mass by myostatin. *Annu. Rev. Cell. Dev. Biol.* **20**:61–
325 86
- 326 Lionikas, A., Kilikevicius, A., Bungler, L., Meharg, C., Carroll, A.M., Ratkevicius, A., et al.
327 2013. Genetic and genomic analyses of musculoskeletal differences between BEH and BEL
328 strains. *Physiol. Genomics*. **45**(20): 940-7. doi:10.1152/physiolgenomics.00109.2013.
- 329 Macaluso, F., Isaacs, A.W., and Myburgh, K.H. 2012. Preferential type II muscle fiber
330 damage from plyometric exercise. *J. Athl. Train.* **47**(4): 414-20. doi:10.4085/1062-6050-
331 47.4.13.
- 332 Matsakas, A., Macharia, R., Otto, A., Elashry, M.I., Mouisel, E., Romanello, V., et al. 2012.
333 Exercise training attenuates the hypermuscular phenotype and restores skeletal muscle
334 function in the myostatin null mouse. *Exp. Physiol.* **97**(1): 125-40.
335 doi:10.1113/expphysiol.2011.063008.

- 336 Matsakas, A., Mouisel, E., Amthor, H., and Patel, K. 2010. Myostatin knockout mice
337 increase oxidative muscle phenotype as an adaptive response to exercise. *J. Muscle Res. Cell.*
338 *Motil.* **31**(2): 111-25. doi:10.1007/s10974-010-9214-9.
- 339 Matsakas, A., Romanello, V., Sartori, R., Masiero, E., Macharia R, Otto, A., et al. 2013. Food
340 restriction reverses the hyper-muscular phenotype and force generation capacity deficit of the
341 myostatin null mouse. *Int. J. Sports. Med.* **34**(3): 223-31. doi:10.1055/s-0032-1312605.
- 342 McHugh, M.P. 2003. Recent advances in the understanding of the repeated bout effect: the
343 protective effect against muscle damage from a single bout of eccentric exercise. *Scand. J.*
344 *Med. Sci. Sports.* **13**(2): 88-97.
- 345 McPherron, A.C., Lawler, A.M., and Lee, S.J. 1997. Regulation of skeletal muscle mass in
346 mice by a new TGF-beta superfamily member. *Nature.* **387**(6628): 83-90.
- 347 Mendias, C.L., Marcin, J.E., Calerdon, D.R., and Faulkner, J.A. 2006. Contractile properties
348 of EDL and *soleus* muscles of myostatin-deficient mice. *J. Appl. Physiol.* **101**(3): 898-905.
- 349 Pizza, F. X., Koh, T.J., McGregor, S.J., and Brooks, S.V. 2002. Muscle inflammatory cells
350 after passive stretches, isometric contractions, and lengthening contractions. *J Appl Physiol*
351 **92**: 1873–1878
- 352 Qaisar, R., Renaud, G., Morine, K., Barton, E.R., Sweeney, H.L., and Larsson, L. 2012. Is
353 functional hypertrophy and specific force coupled with the addition of myonuclei at the
354 single muscle fiber level?. *FASEB J.* **26**(3): 1077-85. doi:10.1096/fj.11-192195.
- 355 Roy, R.R., Hutchison, D.L., Pierotti, D.J., Hodgson, J.A., and Edgerton, V.R. 1991. EMG
356 patterns of rat ankle *extensors* and flexors during treadmill locomotion and swimming. *J.*
357 *Appl. Physiol.* **70**(6): 2522-9.
- 358 Skurvydas, A., Brazaitis, M., Venckūnas, T., and Kamandulis, S. 2011. Predictive value of
359 strength loss as an indicator of muscle damage across multiple drop jumps. *Appl. Physiol.*
360 *Nutr. Metab.* **36**(3): 353-60. doi:10.1139/h11-023.

- 361 Smith, R.C., and Lin, B.K. 2013. Myostatin inhibitors as therapies for muscle wasting
362 associated with cancer and other disorders. *Curr. Opin. Support. Palliat. Care.* 7(4): 352-60.
363 doi:10.1097/SPC.
- 364 Suzuki, K., Totsuka, M., Nakaji, S., Yamada, M., Kudoh, S., Liu, Q., et al. 1999. Endurance
365 exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and
366 muscle damage. *J. Appl. Physiol.* 87(4): 1360-7.
- 367 Tidball, J.G. 1995. Inflammatory cell response to acute muscle injury. *Med. Sci. Sports*
368 *Exerc.* 27(7): 1022-32.
- 369 Tsukada, S., Westwick, J.K., Ikejima K, Sato, N., Rippe, R.A. 2005. SMAD and p38 MAPK
370 signaling pathways independently regulate $\alpha 1(I)$ collagen gene expression in
371 unstimulated and transforming growth factor- β stimulated hepatic stellate cells. *J Biol*
372 *Chem* 280: 10055–10064.
- 373 Wolfe, R.R. 2006. The underappreciated role of muscle in health and disease. *Am. J. Clin.*
374 *Nutr.* 84(3): 475-82.
- 375 Yu, J.G., Liu, J.X., Carlsson, L., Thornell, L.E., Stål, P.S. Re-evaluation of sarcolemma
376 injury and muscle swelling in human skeletal muscles after eccentric exercise. *PLoS One.*
377 2013 Apr 15;8(4):e62056. doi: 10.1371/journal.pone.0062056. Print 2013.
- 378 Warren, G.L., Lowe, D.A., Hayes, D.A., Karwoski, C.J., Prior, B.M. and Armstrong, R.B.
379 1993. Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *J.*
380 *Physiol.* 468, 487-99.
- 381

382 **Table 1.** Age, body mass and muscle mass of *soleus* (SOL) and *extensor digitorum longus*
383 (EDL) muscles in BEH+/+ and BEH mice with the wild type and dysfunctional myostatin,
384 respectively. Values are means and S.D.

	SOL		EDL		
	BEH+/+ (n=25)	BEH (n=22)	BEH+/+ (n=15)	BEH (n=15)	BEH (Older) (n=11)
Age (days)	36.5 ± 5.5	31.7 ± 0.4	36.9 ± 2.7	26.2 ± 1.2	38.5 ± 1.7
Body mass (g)	26.8 ± 2.6	22.9 ± 3.0	27.1 ± 1.8	16.3 ± 1.3	31.4 ± 1.5
Muscle mass (mg)	5.7 ± 0.5	6.0 ± 0.6	7.8 ± 0.4	7.8 ± 0.9	13.8 ± 0.9

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387 **Table 2.** Muscle properties of BEH+/+ and BEH mice with the wild type and dysfunctional
 388 myostatin, respectively. SOL is *soleus* muscle; EDL is *extensor digitorum longus* (EDL). L₀
 389 is optimal muscle length. pCSA is physiological cross-sectional area. Values are means and
 390 S.D. ** P < 0.01 BEH+/+ vs BEH, ## P < 0.01 BEH vs BEH (Older).

	SOL		EDL		
	BEH+/+	BEH	BEH+/+	BEH	BEH (Older)
L ₀ (mm)	12.4 ± 0.9	12.5 ± 0.5	14.3 ± 0.6	12.0 ± 0.5 **	13.0 ± 0.6 **
pCSA (mm ²)	0.84 ± 0.06	0.92 ± 0.08	1.18 ± 0.11	1.40 ± 0.11 **	2.15 ± 0.15 **, ##
Peak isometric force (mN)	173.8 ± 17.6	180.5 ± 13.7	160.3 ± 23.6	145.1 ± 10.5 **	219.9 ± 25.4 **, ##
Specific force (mN/mm ²)	273.8 ± 33.3	271.0 ± 35.2	137.1 ± 23.2	104.3 ± 12.1 *	102.3 ± 9.8, **

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Table 3. Twitch and tetanus contraction and relaxation times in skeletal muscles of BEH+/+ and BEH mice, respectively. SOL is *soleus* muscle; EDL is *extensor digitorum longus* (EDL).

Values are means and S.D.; * P < 0.05, ** P < 0.01 BEH vs BEH+/+, # P < 0.05, ## P < 0.01

BEH (Older) vs BEH.

	SOL		EDL		
	BEH+/+	BEH	BEH+/+	BEH	BEH (Older)
Twitch contraction time (ms)	69.5 ± 8.1	56.9 ± 9.1 **	21.6 ± 0.7	28.3 ± 1.3 **	25.2 ± 1.6 **, #
Twitch relaxation time (ms)	313.9 ± 144.2	304.4 ± 91.5	120.6 ± 23.5	96.2 ± 6.4 *	86.2 ± 8.4 *
Tetanus contraction time (ms)	573.8 ± 54.6	473.0 ± 72.8 **	132.6 ± 9.8	143.0 ± 6.0	125.6 ± 5.7 **, ##
Tetanus relaxation time (ms)	200.7 ± 18.2	163.2 ± 30.7 **	58.7 ± 2.4	68.6 ± 2.4 **	68.4 ± 3.9 **

FIGURE CAPTIONS

Figure 1. Peak isometric force for *soleus* (A) and *extensor digitorum longus* (B) muscles of BEH+/+ and BEH mice with the wild type and mutant myostatin, respectively, during 100 contractions repeated every 10 s. The data for older BEH mice with the mutant myostatin, BEH (Older), is also shown. * $P < 0.05$ for BEH+/+ vs BEH (Older); # $P < 0.05$, ## $P < 0.01$ for BEH vs BEH (Older), respectively. Values are means with S.D.

Figure 2. The total CK efflux at rest and after eccentric exercise from *soleus* (SOL, A) and *extensor digitorum longus* (EDL, B) muscles of BEH and BEH+/+ mice with the mutant and wild type myostatin, respectively. The data for older BEH mice with mutant myostatin, BEH (Older), is also shown (B). * $P < 0.05$, *** $P < 0.001$ for BEH+/+ vs BEH; # $P < 0.001$ for BEH vs BEH (Older) mice. Values are means with S.D.

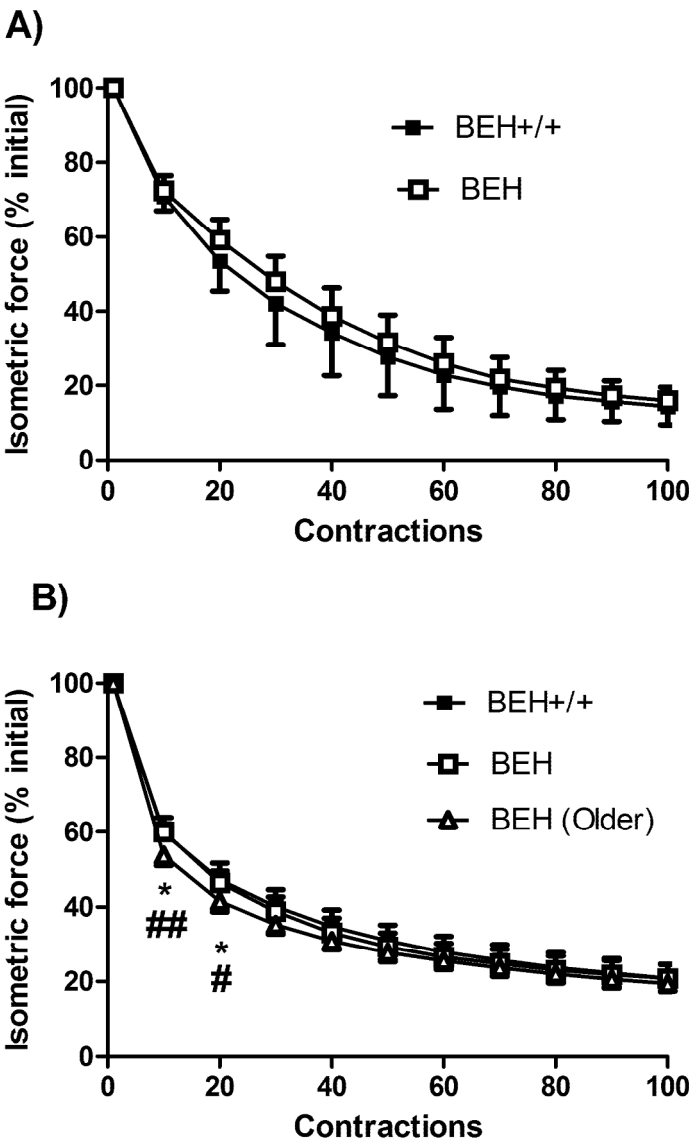


Figure 1. Peak isometric force for soleus (A) and extensor digitorum longus (B) muscles of BEH+/+ and BEH mice with the wild type and mutant myostatin, respectively, during 100 contractions repeated every 10 s. The data for older BEH mice with the mutant myostatin, BEH (Older), is also shown. * $P < 0.05$ for BEH+/+ vs BEH (Older); # $P < 0.05$, ## $P < 0.01$ for BEH vs BEH (Older), respectively. Values are means with S.D. 181x267mm (300 x 300 DPI)

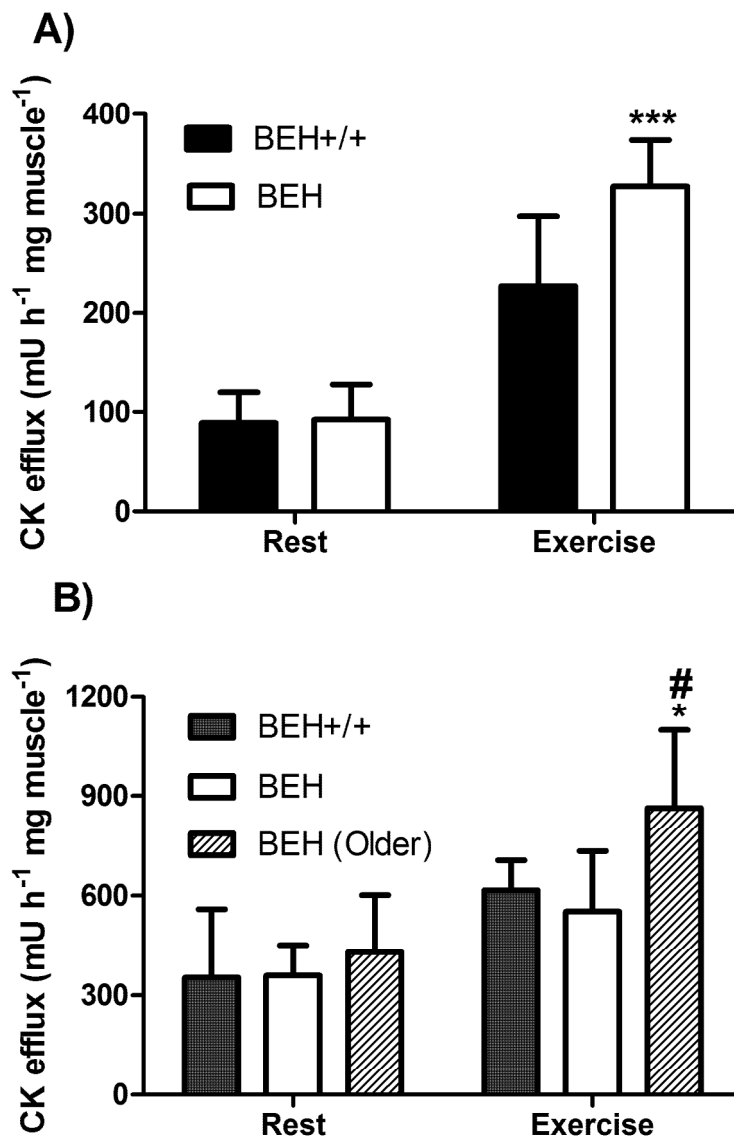


Figure 2. The total CK efflux at rest and after eccentric exercise from soleus (SOL, A) and extensor digitorum longus (EDL, B) muscles of BEH and BEH+/+ mice with the mutant and wild type myostatin, respectively. The data for older BEH mice with mutant myostatin, BEH (Older), is also shown (B). * $P < 0.05$, *** $P < 0.001$ for BEH+/+ vs BEH; # $P < 0.001$ for BEH vs BEH (Older) mice. Values are means with S.D.

180x242mm (300 x 300 DPI)